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Multiyear, Multinational Survey of the Incidence and Global Distribution of Metallo-β-Lactamase-Producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*

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Metallo-β-lactamases (MBLs) hydrolyze all classes of β-lactams except monobactams and are not inhibited by classic serine β-lactamase inhibitors. Gram-negative pathogens isolated from patient infections were collected from 202 medical centers in 40 countries as part of a global surveillance study from 2012 to 2014. Carbapenem-nonsusceptible *Enterobacteriaceae* and *Pseudomonas aeruginosa* were characterized for *bla* genes encoding VIM, IMP, NDM, SPM, and GIM variants using PCR and sequencing. A total of 471 MBL-positive isolates included the following species (numbers of isolates are in parentheses): *P. aeruginosa* (308), *Klebsiella* spp. (85), *Enterobacter* spp. (39), *Proteeae* (16), *Citrobacter freundii* (12), *Escherichia coli* (6), and *Serratia marcescens* (5) and were submitted by sites from 34 countries. Of these, 69.6% were collected in 9 countries (numbers of isolates are in parentheses): Russia (72), Greece (61), Philippines (54), Venezuela (29), and Kuwait, Nigeria, Romania, South Africa, and Thailand (20 to 25 isolates each). Thirty-two different MBL variants were detected (14 VIM, 14 IMP, and 4 NDM enzymes). Seven novel MBL variants were encountered in the study, each differing from a previously reported variant by one amino acid substitution: VIM-42 (VIM-1 [V223I]), VIM-43 (VIM-4 [A24V]), VIM-44 (VIM-2 [K257N]), VIM-45 (VIM-2 [T35I]), IMP-48 (IMP-14 [I69T]), IMP-49 (IMP-18 [V49F]), and NDM-16 (NDM-1 [R264H]). The *in vitro* activities of all tested antibiotics against MBL-positive *Enterobacteriaceae* were significantly reduced with the exception of that of aztreonam-avibactam (MIC_{90}, 0.5 to 1 μg/ml), whereas colistin was the most effective agent against MBL-positive *P. aeruginosa* isolates (>97% susceptible). Although the global percentage of isolates encoding MBLs remains relatively low, their detection in 12 species, 34 countries, and all regions participating in this surveillance study is concerning.

The worldwide dissemination of Gram-negative bacteria producing extended-spectrum β-lactamases (ESBLs) in the late 20th century resulted in a dearth of treatment options and an increase in the therapeutic use of carbapenems (1, 2). In turn, reports of carbapenem-resistant and carbapenemase-producing bacteria became more frequent (3–5). One important group of carbapenemases of special concern is the metallo-β-lactamases (MBLs). These enzymes belong to Ambler class B and require 1 or 2 zinc ions for enzyme activity (6). Many MBLs are chromosomally encoded in environmental bacteria or species that can act as opportunistic pathogens. However, a number of MBLs, including the NDM-type, IMP-type, and VIM-type MBLs, are plasmid encoded and readily transferable among clinically significant bacterial species, including *Klebsiella pneumoniae* and *Escherichia coli* (3). Notably, MBLs are often coproduced with Ambler class A and C serine β-lactamases, including ESBLs and AmpC enzymes (7).

MBLs hydrolyze all β-lactams except monobactams, including aztreonam, and are not inhibited by any of the commercially available β-lactamase inhibitors. Although aztreonam is active against many Gram-negative bacteria, it is inactive against isolates that produce ESBLs, KPC carbapenemases, or plasmid-encoded or stably derepressed, chromosomally encoded AmpC β-lactamases, thereby limiting its potential utility against MBL-producing isolates that also contain one or more of these serine β-lactamases (8). Avibactam is a non-β-lactam β-lactamase inhibitor that is active against Ambler class A and C and some class D (OXA-48) enzymes (9, 10). Aztreonam combined with avibactam has demonstrated activity against *Enterobacteriaceae* that coproduce MBLs and class A or class C β-lactamases (11, 12).

As part of a global surveillance program, the molecular basis of carbapenem resistance in Gram-negative pathogens was investigated in order to determine the incidence of acquired MBLs and the geographic regions in which these β-lactamases are most problematic. This report describes the isolation and regional distribution of MBL-positive *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates collected from 2012 to 2014.

MATERIALS AND METHODS

Nonduplicate bacterial isolates from intra-abdominal, urinary tract, skin and soft tissue, lower respiratory tract, and bloodstream infections were collected from 202 sites in 40 countries located in five major geographic regions (Asia-Pacific, Europe, Latin America, the Middle East-Africa, and North America). A predefined number of isolates of selected bacterial species were collected from each site regardless of antibiotic susceptibility. Organism collection, transport, confirmation of organism identification, susceptibility testing, molecular characterization, quality assurance of

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data, and development and management of a centralized database were coordinated by a central laboratory.

The organism identification of all isolates was confirmed using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker Daltronics, Bremen, Germany). MICs were determined using frozen broth microdilution panels. Aztreonam-avibactam was tested with avibactam at a constant concentration of 4 μg/ml. Panel manufacture, inoculation, incubation, and interpretation were performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (13, 14). Using CLSI breakpoints, all *Enterobacteriaceae* and *P. aeruginosa* isolates that were nonsusceptible to any of the carbapenems tested (meropenem, imipenem, and doripenem) were molecularly characterized for β-lactamase (*bla*) genes encoding MBLs (IMP-, VIM-, NDM-, SPM-type enzymes) and serine β-lactamases (OXA-48-like and KPC-, TEM-, SHV-, CTX-M-, VEB-, PER-, GES-, ACT-, CMY-, DHA-, ACC-, MIR-, MOX-, and FOX-type enzymes) using a combination of the Check-MDR CT101 microarray (Check-Points B.V., Wageningen, the Netherlands) and multiplex PCR assays, followed by full-gene sequencing as reported previously (15). Isolates collected in 2014 were also screened for bla_{GIM} by PCR. Whole-genome sequencing of selected isolates to confirm ambiguous β-lactamase sequences was also performed as described previously (16).

Nucleotide sequence accession numbers. The sequences of seven new variants were deposited in GenBank with accession no. KM087857 (IMP-48), KP681694 (IMP-49), KP071470 (VIM-42), KP096412 (VIM-43), KP681696 (VIM-44), KP681695 (VIM-45), and KP862821 (NDM-16).

RESULTS

A total of 38,266 isolates of *Enterobacteriaceae* and 8,010 isolates of *P. aeruginosa* were collected as part of a global surveillance study from 2012 to 2014. *bla* genes encoding MBL variants were detected in 163 isolates of *Enterobacteriaceae*, which consisted of 11 different bacterial species (12 *Citrobacter freundii*, 1 *Enterobacter aerogenes*, 4 *Enterobacter asburiae*, 34 *Enterobacter cloacae*, 6 *Escherichia coli*, 7 *Klebsiella oxytoca*, 78 *Klebsiella pneumoniae*, 10 *Proteus mirabilis*, 1 *Providencia rettgeri*, 5 *Providencia stuartii*, and 5 *Serratia marcescens* isolates) and 308 isolates of *Pseudomonas aeruginosa*. The MBL-producing isolates were collected in 34 of 40 countries that were sampled and came from all regions involved in the study (Table 1; Fig. 1). No isolates of *Enterobacteriaceae* or *P. aeruginosa* carrying MBL genes were detected in Ireland, Denmark, the Netherlands, Sweden, or Israel. It should be noted that India was not included in this study due to the current restrictions in strain export from that country (17). Among the MBL-positive *Enterobacteriaceae*, 44.2% of isolates carried bla_{NDM}, 39.3% carried bla_{VIM}, and 16.5% carried bla_{IMP}. In contrast, the majority of MBL-positive *P. aeruginosa* isolates carried bla_{VIM} (87.7%), with smaller percentages carrying bla_{IMP} (11.3%) and bla_{NDM} (1.0%). Examples of all three MBL types were found in six species of *Enterobacteriaceae* (*C. freundii*, *E. cloacae*, *K. oxytoca*, *K. pneumoniae*, *P. mirabilis*, and *S. marcescens*) and *P. aeruginosa*. No isolates carrying bla_{SPM} or bla_{GIM} were detected even though isolates from Brazil and Germany were included in the study.

Multiple sequence variants of each MBL type were identified, with 32 variants (14 VIM-, 14 IMP-, and 4 NDM-type enzymes) detected overall (Table 1). Among the *Enterobacteriaceae*, 8 VIM-type, 6 IMP-type, and 4 NDM-type MBLs were detected, with VIM-1 (45 of 64 isolates) and NDM-1 (60 of 72 isolates) being the most commonly found variants of these MBL types. No IMP-type variant predominated, although four variants (IMP-4 [$n = 9$], IMP-8 [$n = 7$], IMP-26 [$n = 5$], and IMP-1 [$n = 4$]) accounted for 25 of 27 IMP-positive isolates. In comparison, 9 VIM-type and 11 IMP-type variants as well as NDM-1 were detected in *P. aeruginosa* isolates, with VIM-2 the most frequently detected VIM-type MBL (240 of 270 isolates). The next most abundant MBL types detected in *P. aeruginosa* were VIM-4 (12 isolates), IMP-7 (8 isolates), and IMP-1 (7 isolates).

Seven novel MBL variants, each differing from a previously reported variant by one amino acid substitution, were identified in six countries as part of this study (Table 2). VIM-42 (VIM-1 [V223I]) and NDM-16 (NDM-1 [R264H]) were found in *K. pneumoniae*, whereas IMP-48 (IMP-14 [I69T]), IMP-49 (IMP-18 [V49F]), VIM-43 (VIM-4 [A24V]), VIM-44 (VIM-2 [K257N]), and VIM-45 (VIM-2 [T35I]) were each found in *P. aeruginosa*. Each new variant was detected in one or two isolates from a single medical center with the exception of IMP-48, which was detected in six isolates collected from two medical centers in Thailand in consecutive years (2013 and 2014). The presumed "progenitor" variant was also detected in each country except Brazil (Table 1). Six of the eight isolates from Thailand (two IMP-48-positive isolates from each medical center and both VIM-45-positive isolates) were collected within a 3-week period in 2014, suggesting possible outbreaks or endemicity. The two NDM-16-positive isolates from Russia were collected 4 months apart at the same medical center (data not shown). Two *P. aeruginosa* isolates carrying VIM-43 were collected from the same center on the same day and were deemed to be duplicate isolates based upon the patient and sample data provided. The second isolate was excluded from analysis.

The distribution of MBL-positive organisms and MBL types varied by region (Table 1; Fig. 1). In Europe and Latin America, the majority of MBL-positive isolates collected were *P. aeruginosa*, whereas relatively equal numbers of MBL-positive *Enterobacteriaceae* and *P. aeruginosa* isolates were collected in Asia-Pacific, the Middle East-Africa, and the United States. Isolates carrying each of the three MBL types were found in all regions except the Middle East-Africa, where no IMP-positive isolates were identified. VIM-positive isolates predominated in Europe and Latin America, comprising 87 to 90% of the MBL-positive organisms collected in these two regions. Three-fourths of the IMP-positive isolates collected globally (77.4%; 48 of 62 global isolates), including all 27 IMP-positive *Enterobacteriaceae*, were found in Asia-Pacific. NDM-positive organisms were found in all regions, with the greatest numbers collected in Philippines, Romania, Nigeria, and Kenya. Notably, the only NDM-positive *P. aeruginosa* isolates identified during this study were collected from two medical centers in Kenya.

The *in vitro* activities of aztreonam-avibactam and comparator antimicrobial agents were determined against the overall collection of *Enterobacteriaceae* and *P. aeruginosa* and subsets of isolates that carried each MBL type (Table 3). The overall MIC_{90} for aztreonam-avibactam against *Enterobacteriaceae* isolates was 0.12 μg/ml, which was >256-fold lower than the MIC_{90} for aztreonam alone (64 μg/ml). Aztreonam-avibactam resulted in MIC_{90}s of 0.5 to 1 μg/ml against MBL-positive isolates, compared to MIC_{90}s of 128 to >128 μg/ml for aztreonam. All MBL-positive *Enterobacteriaceae* isolates were inhibited by ≤8 μg/ml of aztreonam-avibactam. In contrast, the activities of most other tested agents against MBL-positive isolates were greatly reduced. Based on CLSI breakpoints, 75.7% of the overall collection of *Enterobacteriaceae* was susceptible to aztreonam alone, but only 20.8%, 29.6%, and 48.4% of NDM-, IMP-, and VIM-positive subsets, respectively, were susceptible to this single agent due to the coproduction of Ambler

TABLE 1 Distribution of metallo-β-lactamase variants among *Enterobacteriaceae* and *P. aeruginosa* isolates collected from 2012 to 2014

Region	Country	Organism	MBL variant (no.)		
			IMP	NDM	VIM
Europe	Austria	*E. cloacae*			VIM-1 (2)
		P. aeruginosa			VIM-2 (2)
	Belgium	*K. pneumoniae*		NDM-1 (1)	
		P. aeruginosa			VIM-1 (1)
					VIM-2 (8)
	Czech Republic	*P. aeruginosa*	IMP-7 (6)		VIM-2 (2)
	France	*P. aeruginosa*			VIM-2 (1)
	Germany	*P. stuartii*			VIM-1 (1)
		P. aeruginosa	IMP-19 (1)		VIM-2 (3)
					VIM-28 (2)
	Greece	*C. freundii*			VIM-1 (1)
		E. cloacae			VIM-1 (12)
		E. coli			VIM-1 (1)
		K. pneumoniae			VIM-1 (13)
					VIM-26 (1)
		P. mirabilis			VIM-1 (4)
		P. stuartii			VIM-1 (3)
		P. aeruginosa			VIM-2 (24)
					VIM-4 (2)
	Hungary	*C. freundii*			VIM-4 (1)
		K. pneumoniae			VIM-4 (2)
		S. marcescens			VIM-4 (1)
		P. aeruginosa			VIM-4 (5)
					VIM-43 (1)
	Italy	*C. freundii*			VIM-1 (2)
		E. coli			VIM-1 (1)
		K. pneumoniae			VIM-1 (1)
					VIM-42 (1)
		P. aeruginosa			VIM-1 (5)
					VIM-2 (1)
	Poland	*P. aeruginosa*			VIM-2 (1)
	Portugal	*P. aeruginosa*			VIM-2 (4)
					VIM-44 (1)
	Romania	*E. cloacae*		NDM-1 (4)	
		K. oxytoca		NDM-1 (1)	
		K. pneumoniae		NDM-1 (6)	
		S. marcescens		NDM-1 (2)	
		P. aeruginosa			VIM-2 (10)
					VIM-4 (1)
	Russia	*K. pneumoniae*		NDM-1 (3)	
				NDM-16 (2)	
		P. aeruginosa	IMP-1 (1)		VIM-2 (66)
	Spain	*K. oxytoca*			VIM-1 (1)
		P. aeruginosa			VIM-2 (1)
	Turkey	*E. cloacae*			VIM-31 (1)
		K. pneumoniae		NDM-1 (1)	
		S. marcescens			VIM-5 (1)
		P. aeruginosa			VIM-4 (1)
					VIM-5 (1)
	United Kingdom	*K. pneumoniae*		NDM-1 (1)	
		P. aeruginosa			VIM-2 (1)
Asia-Pacific	Australia	*K. pneumoniae*	IMP-4 (1)		
		P. stuartii			VIM-1 (1)
	China[b]	*C. freundii*		NDM-1 (1)	
		E. cloacae	IMP-1 (2)		
			IMP-8 (1)		
		E. coli		NDM-1 (1)	
		K. oxytoca	IMP-4 (3)[a]		
		K. pneumoniae	IMP-4 (2)		
		P. aeruginosa			VIM-2 (1)
	Japan	*K. pneumoniae*	IMP-1 (2)		
		P. aeruginosa	IMP-1 (1)		
			IMP-7 (1)		
	Malaysia	*P. aeruginosa*	IMP-1 (1)		VIM-6 (1)

(Continued on following page)

TABLE 1 (Continued)

Region	Country	Organism	MBL variant (no.)		
			IMP	NDM	VIM
	Philippines	*C. freundii*		NDM-7 (2)	
		E. asburiae		NDM-7 (1)	
		E. cloacae	IMP-4 (1)	NDM-1 (4)	
				NDM-7 (1)	
		K. pneumoniae	IMP-4 (2)	NDM-1 (8)	
			IMP-26 (4)	NDM-7 (2)	
		P. mirabilis	IMP-26 (1)	NDM-1 (1)	
		P. aeruginosa	IMP-1 (1)		
			IMP-26 (5)		VIM-2 (21)
	South Korea	*P. aeruginosa*	IMP-6 (2)		
	Taiwan	*C. freundii*	IMP-8 (3)		
		E. asburiae	IMP-8 (1)		
		E. cloacae	IMP-8 (1)		
		K. pneumoniae	IMP-8 (1)		
		S. marcescens	IMP-47 (1)		
		P. aeruginosa			VIM-2 (1)
	Thailand	*E. asburiae*	IMP-14 (1)		
		E. coli		NDM-1 (1)	
		K. pneumoniae		NDM-1 (3)	
		P. aeruginosa	IMP-1 (2)		
			IMP-7 (1)		VIM-2 (3)
			IMP-14 (1)		VIM-5 (2)
			IMP-48 (6)		VIM-45 (2)
Middle East-Africa	Kenya	*E. asburiae*		NDM-1 (1)	
		K. pneumoniae		NDM-1 (1)	
				NDM-5 (2)	
		P. aeruginosa		NDM-1 (3)	VIM-2 (2)
	Kuwait	*E. cloacae*			VIM-4 (2)
		E. coli		NDM-5 (2)	
		K. pneumoniae		NDM-1 (3)	
		P. aeruginosa			VIM-2 (11)
					VIM-4 (3)
	Nigeria	*E. cloacae*		NDM-1 (1)	
		K. pneumoniae		NDM-1 (9)	
		P. mirabilis			VIM-5 (4)
		P. rettgeri		NDM-1 (1)	
		P. aeruginosa			VIM-2 (3)
					VIM-5 (2)
	South Africa	*K. oxytoca*			VIM-1 (2)
		K. pneumoniae		NDM-1 (3)	
		P. aeruginosa			VIM-2 (20)
Latin America	Argentina	*P. aeruginosa*	IMP-16 (1)		
	Brazil	*P. aeruginosa*	IMP-49 (1)		VIM-2 (2)
	Chile	*P. aeruginosa*			VIM-2 (13)
	Colombia	*P. aeruginosa*			VIM-2 (8)
	Mexico	*C. freundii*			VIM-23 (1)
		E. aerogenes			VIM-23 (1)
		E. cloacae			VIM-23 (2)
		P. aeruginosa	IMP-1 (1)		
			IMP-18 (2)		VIM-2 (1)
	Venezuela	*K. pneumoniae*		NDM-1 (1)	
		P. aeruginosa			VIM-2 (28)
North America	United States	*C. freundii*			VIM-32 (1)
		K. pneumoniae		NDM-1 (2)	
		P. aeruginosa	IMP-13 (1)		VIM-2 (2)

[a] The full gene sequence was determined by whole-genome sequencing.
[b] No isolates were obtained from patients in mainland China in 2014 due to export restrictions.

class A and class C serine β-lactamases. The activities of other β-lactams against MBL-positive isolates were also decreased substantially, with susceptibilities of <8% for ceftazidime and cefepime, <19% for meropenem, and <41% for piperacillin-tazobactam, compared to 76.9%, 78.8%, 97.3%, and 84.7%, respectively, against the overall collection. The activities of agents from other drug classes were also impacted; 96.6% of the overall collection was susceptible to amikacin, but the susceptibility of VIM-positive isolates was 79.7%, and it was further reduced for IMP-positive (66.7%) and for NDM-positive (41.7%) isolates.

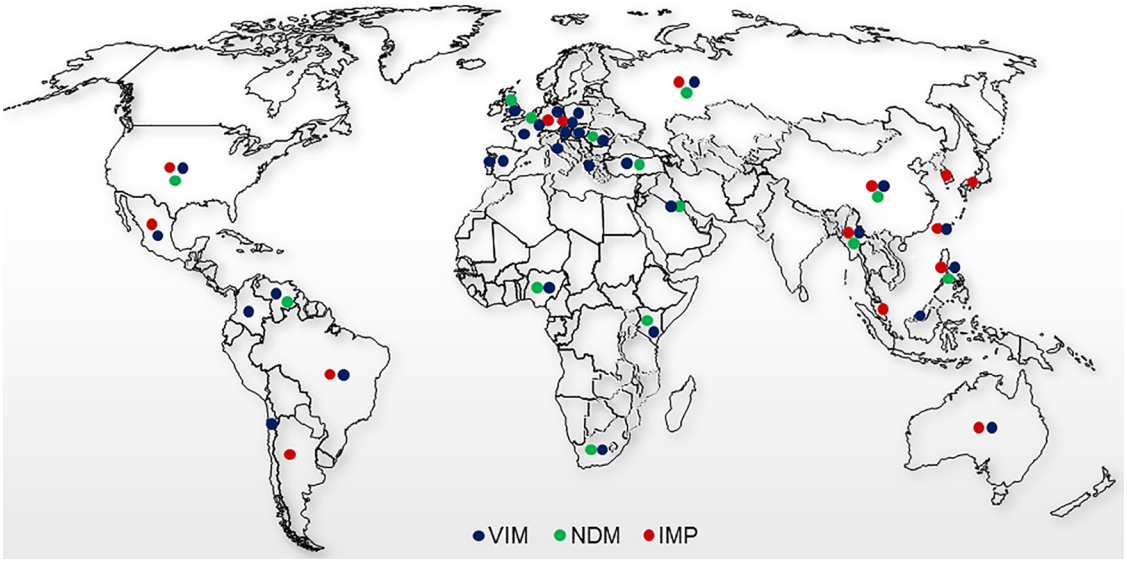

FIG 1 Distribution of metallo-β-lactamase-positive *Enterobacteriaceae* and *P. aeruginosa* collected from 2012 to 2014.

Similarly, the susceptibility of the overall population to levofloxacin was 75.7%, but it was reduced to 55.6% for IMP-positive isolates and 22 to 28% for NDM- and VIM-positive subsets. Tigecycline and colistin retained greater activity against MBL-positive isolates than other comparators. Using FDA breakpoints, 89.0% of MBL-positive isolates were susceptible to tigecycline, compared to 92.9% of all *Enterobacteriaceae* isolates. For colistin, these percentages were 80.4% and 83.2%, respectively, using breakpoints defined by EUCAST (Table 3).

The overall MIC_{90} of aztreonam-avibactam against *P. aeruginosa* was 32 μg/ml (Table 3). Meropenem was also relatively inactive against this species, with only 73.3% of isolates being sus-

TABLE 2 Activities of aztreonam-avibactam and comparator antimicrobial agents tested against isolates carrying new MBL variants collected in 2013 and 2014

Yr collected[c]	Country	Organism	Enzyme content[d]	Source[e]	Ward[f]	MIC (μg/ml)[a,b]								
						ATM	ATM-AVI	FEP	MEM	IPM	AMK	CST	LVX	TGC
2013	Thailand	*P. aeruginosa*[g]	IMP-48	RTI	1	128	128	>16	>8	>8	>32	NA[j]	>4	>8
2014	Thailand	*P. aeruginosa*[g]	IMP-48	IAI	3[i]	64	64	>16	>8	>8	>32	2	>4	>8
2014	Thailand	*P. aeruginosa*[g]	IMP-48	RTI	1	64	64	>16	>8	>8	>32	2	>4	>8
2014	Thailand	*P. aeruginosa*[h]	IMP-48	SSTI	3	64	64	>16	>8	>8	>32	4	>4	>8
2014	Thailand	*P. aeruginosa*[h]	IMP-48	RTI	4	16	2	>16	>8	>8	>32	2	>4	>8
2014	Thailand	*P. aeruginosa*[h]	IMP-48	SSTI	4	128	64	>16	>8	>8	>32	2	>4	>8
2014	Brazil	*P. aeruginosa*	IMP-49	Blood	5	8	8	>16	>8	4	>32	2	1	8
2013	Italy	*K. pneumoniae*	VIM-42, SHV-12	UTI	3	64	0.06	>16	8	8	16	NA	>4	2
2014	Hungary	*P. aeruginosa*	VIM-43[k]	IAI	4	8	8	>16	>8	>8	>32	2	>4	>8
2014	Portugal	*P. aeruginosa*	VIM-44	RTI	4	4	4	8	4	8	>32	2	1	>8
2014	Thailand	*P. aeruginosa*[g]	VIM-45, VEB-1b	RTI	4	>128	64	>16	>8	>8	>32	2	>4	8
2014	Thailand	*P. aeruginosa*[g]	VIM-45, VEB-1b	RTI	1	>128	128	>16	>8	>8	>32	2	>4	>8
2014	Russia	*K. pneumoniae*	NDM-16, CTX-M-15, SHV-OSBL, TEM-OSBL	Blood	1	64	0.12	>16	>8	>8	>32	0.5	>4	0.25
2014	Russia	*K. pneumoniae*	NDM-16, CTX-M-15, SHV-12, TEM-OSBL	Blood	2	>128	0.25	>16	>8	>8	>32	1	>4	0.5

[a] ATM, aztreonam; ATM-AVI, aztreonam-avibactam; FEP, cefepime; MEM, meropenem; IPM, imipenem; AMK, amikacin; CST, colistin, LVX, levofloxacin; TGC, tigecycline.
[b] Colistin was tested with 0.002% polysorbate 80 against isolates collected in 2012 and 2013 per the recommendation of the CLSI *Enterobacteriaceae* Working Group and tested with and without polysorbate 80 against isolates collected in 2014. Values for colistin tested without polysorbate 80 are shown.
[c] Multiple isolates collected from the same site are listed chronologically according to collection date.
[d] OSBL, original-spectrum β-lactamase; includes TEM-1, TEM-2, SHV-1, and SHV-11.
[e] RTI, respiratory tract infection; SSTI, skin and soft tissue infection; blood, bloodstream; UTI, urinary tract infection; IAI, intra-abdominal infection.
[f] Ward types: 1, surgery general; 2, surgery ICU; 3, medicine general, 4, medicine ICU; 5, pediatric ICU.
[g] Isolates were collected from one of two medical centers that contributed 90.9% of MBL-positive isolates from Thailand.
[h] Isolates were collected from one of two medical centers that contributed 90.9% of MBL-positive isolates from Thailand.
[i] Isolate was collected on the day of admittance. All other isolates were collected after ≥48 h of hospitalization.
[j] NA, not available (not determined).
[k] Two *P. aeruginosa* isolates carrying VIM-43 were collected from the same medical center on the same day and were deemed to be duplicate isolates based upon patient and sample data provided by the investigator. The second isolate was excluded from analysis.

TABLE 3 *In vitro* activities of β-lactams and comparators tested against *Enterobacteriaceae* and *P. aeruginosa* encoding metallo-β-lactamases collected from 2012 to 2014

Organism, genotype (no.), and drug[a]	MIC (μg/ml)			% susceptible[b]	
	Range	MIC_{50}	MIC_{90}	CLSI	EUCAST
All *Enterobacteriaceae* (38,266)					
Ceftazidime	≤0.015 to >128	0.25	64	76.9	73.4
Cefepime	≤0.12 to >16	≤0.12	>16	78.8	77.0
Aztreonam	≤0.015 to >128	0.12	64	75.7	73.1
Aztreonam-avibactam[c]	≤0.015 to >128	0.03	0.12	NA[d]	NA
Piperacillin-tazobactam	≤0.25 to >128	2	64	84.7	78.1
Meropenem	≤0.004 to >8	0.03	0.12	97.3	97.7
Amikacin	≤0.25 to >32	2	8	96.6	93.8
Tigecycline	≤0.015 to >8	0.5	2	92.9	82.5
Levofloxacin	≤0.03 to >4	0.06	>4	75.7	73.5
Colistin	≤0.015 to >4	≤0.12	>4	NA	83.2
MBL-positive *Enterobacteriaceae*					
NDM positive (72)					
Ceftazidime	128 to >128	>128	>128	0.0	0.0
Cefepime	1 to >16	>16	>16	0.0	1.4
Aztreonam	≤0.015 to >128	128	>128	20.8	16.7
Aztreonam-avibactam	≤0.015 to 8	0.12	0.5	NA	NA
Piperacillin-tazobactam	32 to >128	>128	>128	0.0	0.0
Meropenem	1 to >8	>8	>8	1.4	2.8
Amikacin	1 to >32	>32	>32	41.7	37.5
Tigecycline	0.06 to 8	1	4	87.5	61.1
Levofloxacin	0.06 to >4	>4	>4	22.2	12.5
Colistin	≤0.015 to >4	≤0.12	>4	NA	86.1
IMP positive (27)					
Ceftazidime	64 to >128	>128	>128	0.0	0.0
Cefepime	8 to >16	>16	>16	0.0	0.0
Aztreonam	0.06 to >128	64	>128	29.6	29.6
Aztreonam-avibactam	0.03 to 4	0.25	1	NA	NA
Piperacillin-tazobactam	0.5 to >128	128	>128	40.7	25.9
Meropenem	0.5 to >8	4	>8	18.5	40.7
Amikacin	1 to >32	2	>32	66.7	59.3
Tigecycline	0.12 to 8	1	2	96.3	77.8
Levofloxacin	0.06 to >4	2	>4	55.6	40.7
Colistin	0.03 to >4	≤0.12	>4	NA	88.9
VIM positive (64)					
Ceftazidime	0.25 to >128	>128	>128	6.3	6.3
Cefepime	≤0.12 to >16	>16	>16	7.8	7.8
Aztreonam	≤0.015 to >128	8	128	48.4	42.2
Aztreonam-avibactam	≤0.015 to 2	0.12	1	NA	NA
Piperacillin-tazobactam	1 to >128	>128	>128	6.3	1.6
Meropenem	0.25 to >8	8	>8	14.1	25.0
Amikacin	1 to >32	8	>32	79.7	59.4
Tigecycline	0.25 to >8	1	4	87.5	60.9
Levofloxacin	0.06 to >4	>4	>4	28.1	23.4
Colistin	0.03 to >4	≤0.12	>4	NA	70.3
All *P. aeruginosa* (8,010)					
Ceftazidime	0.06 to >128	2	64	77.4	77.4
Cefepime	≤0.12 to >16	4	16	78.6	78.6
Aztreonam	≤0.015 to >128	8	32	61.4	3.4
Aztreonam-avibactam[c]	≤0.015 to >128	8	32	NA	NA
Piperacillin-tazobactam	≤0.25 to >128	8	>128	69.1	69.1
Meropenem	≤0.06 to >8	0.5	>8	73.3	73.3
Amikacin	≤0.25 to >32	4	16	90.2	85.1
Levofloxacin	≤0.03 to >4	0.5	>4	71.7	63.2
Colistin	≤0.06 to >8	0.5	1	99.5	99.7

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TABLE 3 (Continued)

Organism, genotype (no.), and drug[a]	MIC (μg/ml)			% susceptible[b]	
	Range	MIC_{50}	MIC_{90}	CLSI	EUCAST
MBL-positive *P. aeruginosa*					
NDM positive (3)					
Ceftazidime	>128			0.0	0.0
Cefepime	>16			0.0	0.0
Aztreonam	>128			0.0	0.0
Aztreonam-avibactam	16 to >128			NA	NA
Piperacillin-tazobactam	>128			0.0	0.0
Meropenem	>8			0.0	0.0
Amikacin	>32			0.0	0.0
Levofloxacin	>4			0.0	0.0
Colistin	≤0.06 to 2			100	100
IMP positive (35)					
Ceftazidime	64 to >128	>128	>128	0.0	0.0
Cefepime	>16	>16	>16	0.0	0.0
Aztreonam	4 to >128	32	128	17.1	0.0
Aztreonam-avibactam	2 to 128	32	64	NA	NA
Piperacillin-tazobactam	4 to >128	128	>128	14.3	14.3
Meropenem	4 to >8	>8	>8	0.0	0.0
Amikacin	4 to >32	32	>32	40.0	28.6
Levofloxacin	1 to >4	>4	>4	5.7	5.7
Colistin	0.12 to 4	0.5	2	97.1	100
VIM positive (270)					
Ceftazidime	2 to >128	64	>128	3.0	3.0
Cefepime	8 to >16	>16	>16	4.4	4.4
Aztreonam	0.5 to >128	16	64	27.8	0.4
Aztreonam-avibactam	0.25 to >128	16	32	NA	NA
Piperacillin-tazobactam	16 to >128	64	>128	3.7	3.7
Meropenem	1 to >8	>8	>8	2.2	2.2
Amikacin	0.5 to >32	>32	>32	16.7	11.1
Levofloxacin	0.25 to >4	>4	>4	6.3	5.2
Colistin	≤0.06 to 2	0.5	1	100	100

[a] Colistin was tested with 0.002% polysorbate 80 against isolates collected in 2012 and 2013 per the recommendation of the CLSI *Enterobacteriaceae* Working Group and tested with and without polysorbate 80 against isolates collected in 2014. Values for colistin tested with 0.002% polysorbate 80 are shown.
[b] CLSI susceptibilities were defined by CLSI document M100-S25 (14). Tigecycline susceptibilities in the CLSI category were defined by the FDA (54). EUCAST susceptibilities were defined by *Breakpoint Tables for Interpretation of MICs and Zone Diameters*, version 5.0 (55).
[c] Aztreonam-avibactam was tested at a fixed concentration of 4 μg/ml of avibactam.
[d] NA, not applicable (no breakpoint defined).

ceptible to meropenem. Amikacin and colistin were the only agents for which a susceptibility of >90% within the overall collection was found. Aztreonam-avibactam showed a modest (2-fold) increase in activity against VIM- and IMP-positive isolates of *P. aeruginosa* compared to that of aztreonam alone (MIC_{90}s of 32 to 64 μg/ml). Colistin was the only agent that retained significant activity against IMP-, VIM-, and NDM-positive subsets of *P. aeruginosa* (>97% susceptible).

A total of 98.7% of MBL-positive isolates also carried 1 to 5 genes encoding serine β-lactamases from Amber class A, C, or D detected by PCR or intrinsic chromosomally encoded AmpC and ESBL enzymes common to *Citrobacter* spp., *Enterobacter* spp., *Providencia* spp., *Serratia* spp., *P. aeruginosa*, and *K. oxytoca* that were presumed to be present, though this was not confirmed by molecular methods (Table 4). Of the MBL-positive isolates, only 24 were negative for any additional serine *bla* gene or carried only an original-spectrum β-lactamase (OSBL) that would not hydrolyze aztreonam. A small number of MBL-positive isolates also carried genes encoding KPC or OXA-48 carbapenemases, with or without additional serine β-lactamases. One *P. aeruginosa* isolate collected in Chile carried VIM-2 and KPC-2. Four *K. pneumoniae* isolates from Greece carried VIM-1 and KPC-2, three of which also coproduced additional class A and class C enzymes, and two *K. oxytoca* isolates from China carried IMP-4, KPC-2, and SHV-12. Three *E. cloacae* isolates carried VIM-type enzymes (VIM-4, Kuwait; VIM-31, Turkey) in combination with OXA-48. Three *K. pneumoniae* isolates collected in Romania carried NDM-1, OXA-48, and CTX-M-15, whereas one *K. pneumoniae* isolate from Belgium carried NDM-1 and OXA-232.

In contrast, the percentages of MBL-positive isolates that carried genes encoding ESBL and AmpC enzymes (9.8%), only ESBLs (11.9%), or only AmpC β-lactamases (70.3%) were much higher. NDM-type and CTX-M-type β-lactamases were detected in 50 isolates of *K. pneumoniae*, *E. cloacae*, *C. freundii*, *E. coli*, and *S. marcescens*, 47 of which also carried CTX-M-15. Ten of 47 isolates carried an additional ESBL (SHV-type enzyme or CTX-M-27), and 2 carried additional plasmid-mediated AmpC enzymes. VIM-1 was cocarried with SHV-type, CTX-M-14, or VEB-1 ESBLs in *C. freundii*, *E. cloacae*, *P. mirabilis*, *P. stuartii*, and *K.*

TABLE 4 Cocarriage of metallo-β-lactamases and serine β-lactamases in *Enterobacteriaceae* and *P. aeruginosa* collected from 2012 to 2014[a]

MBL and serine β-lactamase	Organism	No. of isolates	Molecular variant(s)
MBL + KPC	*K. pneumoniae*	1	VIM-1, KPC-2
MBL + KPC + ESBL + AmpC + OSBL[b]	*K. pneumoniae*	2	VIM-1, KPC-2, SHV-12, CMY-13, TEM-OSBL
MBL + KPC + ESBL	*K. oxytoca*	2	IMP-4, KPC-2, SHV-12
MBL + KPC + AmpC	*P. aeruginosa*[c]	1	VIM-2, KPC-2
MBL + KPC + AmpC + OSBL	*K. pneumoniae*	1	VIM-1, KPC-2, MOX-1, SHV-OSBL
MBL + OXA carbapenemase + ESBL + AmpC + OSBL	*E. cloacae*[c]	1	VIM-4, OXA-48, SHV-12, CMY-4, TEM-OSBL
MBL + OXA carbapenemase + ESBL + OSBL	*K. pneumoniae*	3	NDM-1, OXA-48, CTX-M-15, SHV-OSBL
MBL + OXA carbapenemase + AmpC	*E. cloacae*[c]	1	VIM-4, OXA-48, CMY-4
		1	VIM-31, OXA-48
MBL + OXA carbapenemase + OSBL	*K. pneumoniae*	1	NDM-1, OXA-232, SHV-OSBL
MBL + ESBL + AmpC ± OSBL	*C. freundii*[c]	1	IMP-8, SHV-12, TEM-OSBL
		2	VIM-1, SHV-12
		1	VIM-4, CTX-M-15, TEM-OSBL
		1	NDM-1, CTX-M-3, SHV-12, TEM-OSBL
	E. asburiae[c]	1	IMP-8, SHV-12, TEM-OSBL
		1	NDM-1, VEB-1, TEM-OSBL
	E. cloacae[c]	1	IMP-8, SHV-12, TEM-OSBL
		1	IMP-8, CTX-M-22, TEM-OSBL
		1	VIM-1, CTX-M-14, TEM-OSBL
		1	NDM-1, CTX-M-15
		4	NDM-1, CTX-M-15, TEM-OSBL
		2	NDM-1, CTX-M-15, SHV-31, TEM-OSBL
	K. oxytoca[d]	1	VIM-1, ACC-1
		1	NDM-1, ACC-1, TEM-OSBL
	K. pneumoniae	1	IMP-26, CTX-M-15, DHA-1, SHV-OSBL, TEM-OSBL
		1	NDM-1, CTX-M-15, CMY-6, SHV-OSBL, TEM-OSBL
		1	NDM-1, CTX-M-15, CMY-6, DHA-type, SHV-OSBL, TEM-OSBL
	P. stuartii[c]	1	VIM-1, SHV-5, TEM-OSBL
		3	VIM-1, SHV-5, VEB-1, TEM-OSBL
	S. marcescens[c]	2	NDM-1, CTX-M-15, TEM-OSBL
	P. aeruginosa[c]	1	VIM-2, SHV-2A
		2	VIM-2, SHV-12
		1	VIM-2, GES-1
		4	VIM-2, PER-1
		1	VIM-2, VEB-1b
		1	VIM-2, VEB-1
		1	VIM-4, SHV-12
		1	VIM-5, PER-1
		2	VIM-5, VEB-14[e]
		2	VIM-45, VEB-1b[f]
		2	NDM-1, VEB-1a
MBL + ESBL ± OSBL	*E. coli*	1	NDM-1, CTX-M-3
		1	NDM-1, CTX-M-27
	K. oxytoca[d]	1	IMP-4[f]
		2	VIM-1
	K. pneumoniae	1	IMP-1, CTX-M-3, SHV-OSBL
		2	IMP-4, CTX-M-15, SHV-OSBL
		1	IMP-4, CTX-M-15, SHV-OSBL, TEM-OSBL
		1	IMP-26, CTX-M-15, SHV-28
		1	IMP-26, CTX-M-15, SHV-OSBL
		1	IMP-26, CTX-M-15, SHV-OSBL, TEM-OSBL
		2	VIM-1, SHV-12
		2	VIM-4, CTX-M-15, SHV-OSBL, TEM-OSBL
		1	VIM-26, SHV-5
		1	VIM-42, SHV-12
		1	NDM-1, CTX-M-15
		9	NDM-1, CTX-M-15, SHV-OSBL
		16	NDM-1, CTX-M-15, SHV-OSBL, TEM-OSBL
		1	NDM-1, CTX-M-15, SHV-12
		1	NDM-1, CTX-M-15, SHV-12, TEM-OSBL
		1	NDM-1, CTX-M-15, SHV-55, TEM-OSBL
		1	NDM-1, CTX-M-15, SHV-134, TEM-OSBL
		1	NDM-1, CTX-M-15, CTX-M-27, TEM-OSBL
		1	NDM-5, CTX-M-15, SHV-OSBL, TEM-OSBL
		2	NDM-7, CTX-M-15, SHV-12, TEM-OSBL

(Continued on following page)

TABLE 4 (Continued)

MBL and serine β-lactamase	Organism	No. of isolates	Molecular variant(s)
		1	NDM-16, CTX-M-15, SHV-OSBL, TEM-OSBL
		1	NDM-16, CTX-M-15, SHV-12, TEM-OSBL
	P. mirabilis	1	VIM-1, SHV-5, VEB-1, TEM-OSBL
		1	VIM-1, VEB-1, TEM-OSBL
MBL + AmpC ± OSBL	*C. freundii*[c]	2	IMP-8, TEM-OSBL
		1	VIM-1
		1	VIM-23
		1	VIM-32
		1	NDM-7, DHA-1
		1	NDM-7, TEM-OSBL
	E. aerogenes[c]	1	VIM-23
	E. asburiae[c]	1	IMP-14
		1	NDM-7
	E. cloacae[c]	2	IMP-1
		1	IMP-4
		6	VIM-1
		7	VIM-1, TEM-OSBL
		2	VIM-23
		2	NDM-1, DHA-1, TEM-OSBL
		1	NDM-7
	E. coli	1	VIM-1, ACT-24, TEM-OSBL
		1	NDM-5, CMY-42
		1	NDM-5, CMY-42, TEM-OSBL
	P. mirabilis	1	IMP-26, DHA-1
		2	VIM-1, CMY-16, TEM-OSBL
	P. rettgeri[c]	1	NDM-1, TEM-OSBL
	P. stuartii[c]	1	VIM-1, CMY-2
	S. marcescens[c]	1	IMP-47, TEM-OSBL
		1	VIM-4
		1	VIM-5
	P. aeruginosa[c]	6	IMP-1
		1	IMP-1, TEM-OSBL
		2	IMP-6
		8	IMP-7
		1	IMP-13
		1	IMP-14
		1	IMP-16
		2	IMP-18
		1	IMP-19
		5	IMP-26
		6	IMP-48
		1	IMP-49
		6	VIM-1
		228	VIM-2
		1	VIM-2, TEM-OSBL
		11	VIM-4
		2	VIM-5
		1	VIM-6
		2	VIM-28
		1	VIM-43
		1	VIM-44
		1	NDM-1
MBL + OSBL	*E. coli*	1	VIM-1, SHV-OSBL
	K. pneumoniae	1	IMP-1, SHV-OSBL
		1	IMP-4, SHV-OSBL, TEM-OSBL
		1	IMP-4, TEM-OSBL
		1	IMP-8, SHV-OSBL
		8	VIM-1, SHV-OSBL
		4	NDM-1, SHV-OSBL
		1	NDM-5, SHV-OSBL

[a] Serine β-lactamases were not detected by PCR in 6 MBL-positive *Enterobacteriaceae* isolates (1 *K. pneumoniae* isolate and 5 *P. mirabilis* isolates).
[b] OSBL, original spectrum β-lactamase; includes TEM-1, TEM-2, SHV-1, and SHV-11.
[c] Presumed to also carry the intrinsic chromosomally encoded AmpC β-lactamase common to this species.
[d] Presumed to also carry the intrinsic chromosomally encoded ESBL common to this species.
[e] S. Lahiri (formerly of AstraZeneca Pharmaceuticals), personal communication.
[f] The full gene sequence was determined by whole-genome sequencing.

pneumoniae isolates, whereas VIM-4 plus CTX-M-15 was detected in *C. freundii* and *K. pneumoniae*. IMP-8 along with SHV-12 or CTX-M-22 was detected in *C. freundii* and *Enterobacter* spp., and IMP-26 plus CTX-M-15 was found in *K. pneumoniae*. Sixteen VIM-positive *P. aeruginosa* isolates also contained PER-, VEB-, SHV-, and GES-type ESBLs, and two isolates contained NDM-1 and VEB-1a.

DISCUSSION

This 2012-2014 global surveillance program provided a contemporary perspective on the species, regions, and types of MBL-producing pathogens that are a significant concern among patient infections. Although this surveillance program was not designed to be a prevalence study, the incidence and distribution of MBL-positive isolates resembled those reported by others, with VIM-type MBLs predominating in Europe and Latin America but found globally, IMP-type enzymes most common in Asia-Pacific, and NDM-type enzymes found in all regions but in higher numbers in countries in the Balkan region and the Middle East-Africa (18–22). It is unfortunate that medical centers in India did not contribute isolates to this study, as a high prevalence of MBL-producing isolates from the Indian subcontinent has previously been reported (23, 24). While isolates carrying bla_{SPM} have been reported to be endemic in Brazil, surprisingly, no isolates carrying bla_{SPM} were detected in that country during the 3 years of this study (25). Multiple MBL-positive isolates were detected in Russia and Greece, and the prevalence and spread of MBL-positive organisms within and from these countries have been reported previously (18, 26–28). In this study, the large number of MBL-positive organisms isolated in Philippines, which included 5 different species of *Enterobacteriaceae* and *P. aeruginosa* carrying genes for all three MBL types, exceeded those in previous reports and might suggest a strong potential for further spread in diverse geographic regions (29, 30).

Some variants were detected in one or several species of *Enterobacteriaceae*, whereas others were found in both *Enterobacteriaceae* and *P. aeruginosa* isolates, suggesting that these variants were encoded on mobilizable elements with large host ranges. bla_{VIM} and bla_{IMP} are commonly found as gene cassettes within class 1 integrons that can be mobilized by transposition or plasmid conjugation (3, 21, 31). bla_{NDM-1} is often flanked by IS*Aba125* and ble_{MBL} and has been found on both narrow- and broad-host range plasmids belonging to at least 8 incompatibility groups, many of which are readily transmissible within and between species (19). These three MBL types have been identified in multiple lineages of *Enterobacteriaceae* species and *P. aeruginosa* (19, 21). MBLs can also spread clonally, and some have been associated with successful high-risk sequence types (STs), such as *P. aeruginosa* ST111 and ST235 and *E. coli* ST131 and ST405 (32–35).

Although only a small number of isolates coproducing MBLs and serine carbapenemases were identified during this study, similar isolates have been reported by others, though they appear to be rare. Isolates producing KPC-2 and VIM-type MBLs (36–43) and *K. pneumoniae* carrying NDM-1 and OXA-48 or OXA-232 (44–47) have been reported previously. Although *E. cloacae* isolates carrying VIM-31, VIM-4 and CMY-4, or OXA-48 are known, this is the first report of isolates carrying OXA-48 in combination with those VIM types (48–50).

Infections caused by MBL-positive isolates pose a grave health challenge. Genes encoding MBLs have disseminated to several difficult-to-treat ESKAPE pathogens and to species that are naturally resistant to colistin (*Proteeae* and *Serratia* spp.) and tigecycline (*Proteeae* and *P. aeruginosa*) (51, 52). Furthermore, resistance mechanisms against non-β-lactam classes of antimicrobials, such as aminoglycosides and fluoroquinolones, are often transferred with *bla* genes encoding MBLs (19, 31, 53). As a result, many MBL-containing isolates are truly multidrug resistant, with limited options for treatment. Although aztreonam is stable to hydrolysis by MBLs, the majority of MBL-positive isolates coproduce one or more serine β-lactamases that can hydrolyze aztreonam and are therefore resistant to this agent. Combining aztreonam with avibactam would alleviate this issue, and the activity of aztreonam-avibactam against MBL-positive *Enterobacteriaceae*, including isolates harboring MBLs and serine carbapenemases, is noteworthy. However, colistin appears to be the only agent active against MBL-producing *P. aeruginosa*. The greatly diminished susceptibility of MBL-positive isolates to most antimicrobials and limited therapeutic options currently available demand continued monitoring and research into the development of new inhibitors of this formidable class of enzymes.

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